Production of Ethanol from *Lantana camara* L. Leaves Using *Saccharomyces cerevisiae*

Sara Getachew Amenu*, Meseret Chimdessa (PhD) and Manikandan Muthuswamy (PhD)

Haramaya University, ETHIOPIA. sarahgetachew22@gmail.com

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Abstract: Production of bio-ethanol from lignocellulose materials is an alternative means of fulfilling the demand for fuel. Lantana camara is an invasive weed, which can serve as a source of lignocellulose material for bioethanol production. This study was conducted with the objective of evaluating bio-ethanol production from leaves of L.camara using yeast (Saccharomyces cescerevisiae) in batch fermentation. L.camara leaves in different concentrations (10g, 20g, 30g and 40g) pre-treated with dilute sulfuric acid or untreated and supplemented with 0.5 or 1% of yeast as inoculums were treatments. Ethanol production, amount of reducing sugar and cell density were parameters measured at 4th day interval starting from incubation period. Results showed that Ethanol production, amount of reducing sugar and cell density were parameters measured at 4th day interval starting from incubation period. Results showed that reducing sugar was observed starting from the 4th day of fermentation, increase but Concomitant with ethanol production, cell density were observed in the same pattern its amount peaked on the 8th day of fermentation, and declined afterwards Compared to untreated substrates, acid pre-treated substrates yielded more bio-ethanol. Overall, this study showed that acid pre-treatment, inoculum concentration, fermentation period and substrate concentration affect the amount of bio-ethanol production.
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1. Introduction

Ethanol is a liquid alcohol composed of oxygen, hydrogen and carbon and is obtained from the fermentation of sugar or converted starch contained in grains and other agricultural or agro-forest feed stocks (Prescott and Dunn, 1959). Species of yeast (*Saccharomyces cerevisiae*) metabolizes sugar in the absence of oxygen and produces ethanol and carbon dioxide. Ethanol is an important industrial solvent and chemical feed stock for the synthesis of pharmaceuticals, detergents, adhesives, plastics and host for other chemicals (Prescott and Dunn, 1959). Apart from methane and hydrogen, ethanol is also being used as bio-fuel substituting other non-renewable energy sources such as petroleum and coal (Smith, 2007) that as a result, bio-ethanol production is currently considered as one of the important commercial activities of many countries as it serves as alternative energy source like.

Raw materials used for the production of ethanol via fermentation are conveniently classified under three types namely: sugar, starches, and cellulose materials. Sugars (e.g., sugar cane, sugar beets, molasses, and fruits) can be converted to ethanol directly. Starch (e.g., grains, potatoes, root crops) must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds. Cellulose (e.g., wood, agricultural residues, waste sulfite liquor from pulp and paper mills) must likewise be converted to sugars generally by the action of mineral acids. Once simple sugars are formed, enzymes from yeast can readily ferment them to ethanol (Dickinson, 1999).

Depletion of crude oil with increasing energy demands creates attention to utilize lignocelluloses materials for low-cost ethanol production. Ethanol, a high octane contain oxygenated fuel is known to run combustion engines at higher compression ratios providing superior performance (Dickinson, 1999). To make the lignocelluloses accessible to the fermenting microorganisms, the biomass should be first pre-treated under various conditions. *S. cerevisiae* is able to ferment a wider range of sugars than other microorganism (Dominguez *et al.,* 1993). Among the pentose fermenting yeasts, *S. cerevisiae* is able to ferment xylose rapidly with a high ethanol yield and comparatively produces less xylitol (Dominguez *et al.,* 1993).

Lantana camara L. (Verbenaceae) widespread and diverse distribution is a reflection of its wide ecological tolerances. The species occurs in varied habitats ranging from open unshaded regions which include wastelands, rainforest edges, beachfronts, and forests disturbed by activities such as fire or logging (Thakur *et al*., 1992, Rishi, 2009). The species also

thrive well in disturbed areas which includes roadside, railway tracks and canals (Sharma *et al*., 2005; Kohli *et al*., 2008; Dogra *et al*., 2010). Anthropogenic activity further aggravates the invasion and allows it to spread (Day *et al*., 2003). Based on diversity, broad geographic expansion and wide ecological tolerance are so inbuilt in species, that the Invasive Species Specialist Group (ISSG) considers it as among 100 of the "World's Worst" invaders (ISSG 2001). A considerable amount of laboratory works has been in progress for bioconversion of various lignocellulose materials into ethanol using sequential steps of hydrolysis, scarification and fermentation. However, there is no any reported work for bioconversion of *L.* into fuel ethanol production in Ethiopia. Therefore, this research was initiated to produce bio-ethanol from *L. camara* leaves with the following general and specific objectives.

General objective:

❖ To produce bio-ethanol from *Lantana camara* leaves by batch culture using *S. cerevisiae* as fermenting microbe.

Specific objectives:

- To identify the optimum substrate concentration for bio-ethanol production from *L. camara* leaves;
- To determine the effect of inoculum (yeast) concentration on the rate of ethanol production from *L. camara* leaves;
- To determine cell density and reducing sugar concentrations at different fermentation time;
- To assess the effect of pre-treatment on the rate of ethanol production from *L. camara* leaves.

2. Materials and Methods

2.1 Description of the Study Area

The experiment was conducted in Botanical Science laboratory, Department of Biology at Haramaya University, which is, located at latitude of 9º26' N, longitude of 42º03'E and altitude of 1980 m.a.s.1 (FAO, 1990).

2.2. Substrate Preparation

Fresh *L. camara* leaves were collected from Haramaya University campus. The leaves were washed using distilled water, and chopped and dried in oven at 60 ± 3^{O}C for 24 h prior to pre-treatment. pre-treatment different substrate amount (10g, 20g, 30g and 40g) was then be pretreated with 10ml at 0.5% (v/v) H2SO4 add to each gram of substrate and mixture was shaken and autoclaved at 11-12 bars for approximately 15 minutes, 121c^{o} autoclaving (Karimi*et al*., 2006) and the solid residue was sieved and stored in polypropylene for subsequent use (Singh *et al*., 2014).

2.3. Inoculums Preparation

Inoculums were prepared from dried baker's yeast, *Saccharomyces cerevisiae* that was purchased from Neway plc. For preparing 0.5% and 1% inoculums concentration, 0.5 g and 1 g of yeast *(S. cerevisiae)* was dissolved in 99.5 and 99ml of distilled water respectively. For fermentation of each substrate, 10ml of the solutions was used as inoculums concentration for batch fermentation process (Dhopes *et al.*, 2001).

2.4. Preparation of Nutrient Solution

Nutrient supplements were prepared by adding 0.1 g KH_2PO_4, 0.5 g $CaCl_2$, 0.5 g $MgSO_4$, 0.1 g Na_2SO_4 and 0.1 g $(NH_4)_2SO_4$ per liter of the culture solution (Abouzeid and Reddy, 1986).

2.5. Experimental Design and Fermentation

Table 2. Treatment combinations for bioethanol production from *lantana camara* leaves**.**

Samples	Substrate (gm)	Volume of Nutrient Solution (ml)	Inoculums Concentration
A	10 treated 10 untreated	100	0.5%
B	10 treated 10 untreated	100	1%
C	20 treated 20 untreated	100	0.5%
D	20 treated 20 untreated	100	1%
E	30 treated 30 untreated	100	0.5%
F	30 treated 30 untreated	100	1%
G	40 treated 40 untreated	100	0.5%
H	40 treated 40 untreated	100	1%

The experimental design was CRD factorial with eight treatment combinations in three replications for each treatment and control. Batch fermentation was carried out on *Lantana camara* leaves as substrate and using baker's yeasts as fermenter in 500ml. Erlenmeyer flasks. Different amounts (10, 20, 30 and 40g) of acid-treated substrate and inoculums concentrations (0.5% and 1%) were treatments (Table 3). Acid-untreated substrates with inoculum were controls. In all cases, the pH was adjusted to 4.5 with buffer solution. The fermentation process was allowed for 16 days at 30^{o}C. The production of bio-ethanol and other parameters were estimated at 4 days interval starting from the beginning of fermentation (Akin *et al.,* 2005).

2.6. Determination of Cell Density (Biomass)

Cell density was measured on the 4th, 8th, 12th and 16thday of fermentation using spectrophotometer (Humas Think HS 3300, Korea) at 600 nm absorbance (Summer *et al*., 2004). Dry weight method of cell measurement was used. The cell in the broth sample was separated by centrifugation and the wet weight of the culture was measured immediately and allowed to dry in oven at 100^{o}C for six hours. The difference in weight was calculated and expressed the dry weight in mg /ml. Then the sample was diluted and measured the absorbance of it with a spectrophotometer at 600 nm. The calibration curve to relate the absorbance (0.5, 0.25, 0.1) with cell dry weight (5.0g/ml, 2.5g/ml, 1.25g/ml) was then be generated.

2.7. Estimation of total reducing sugar

The amount of reducing sugar in the fermenting sample broth was estimated spectrophoto metrically following the method used by Nelson (1944) and Somogyi (1945) using D-glucose as standard. A fermenting sample (0.05ml) was mixed with 0.35ml citrate buffer (pH=6.5) and 0.6ml of Dinitrosalicylic acid (DNS) and then the mixture was boiled for 5 minutes immediately to stop the reaction. Then the absorbance was measured at 540nm using spectrophotometer (Benn et al., 1971). The amount of reducing sugar in the sample was calculated by relating absorbance of different concentrations of glucose (0,2g/ml,0.4g/ml,0.6g/ml,0.8 g/ml 1 ml) on standard curve (Sadasivam and Manickam, 1996).

2.8. Quantitative Estimation of Bio-ethanol

One ml of the fermented sample was taken into 500 ml Pyrex distillation flask containing 30 ml of distilled water and distilled. The distillate was then collected in 50 ml flask containing 25 ml of potassium dichromate solution (33.76 g of K2Cr2O7 dissolved in 400 ml of distilled water with 325 ml of sulphuric acid) and volume raised to 1 litter. About 20 ml of distillate was then collected in each sample and the flasks was kept in a water bath maintained at 6o°C for 20 minutes. The flasks were cooled to room temperature and add distilled water until the volume raised to 50 ml. the volume raised to 50 ml. Five ml of this solution was diluted with 5 ml of distilled water for measuring the optical density at 600 nm using spectrophotometer (Caputi *et al.*, 1968). A standard curve was prepared under similar set of conditions by using standard solution of ethanol containing 0 to 20% (v/v) ethanol in distilled water and then ethanol content of each sample was estimated by relating absorbance of known ethanol concentration from the standard curve (Yoswathana and Phuriphipat, 2010).

2.9. Data analysis

The data was analyzed using (SPSS version 20). Duncan's multiple range tests and least significant difference (LSD) test was used to identify significant differences among treatment means. P< 0.05 was considered significant in all cases.

3. Results and Discussion

3.1. Effect of acid pre-treatment, substrate and inoculums concentration and Fermentation Period on Ethanol Production

This study showed that the amount of ethanol production is affected by substrate concentration, fermentation duration, inoculums concentration and acid pre-treatments. With respect to fermentation duration, ethanol production was observed on the 4th day of fermentation in both acid pre-treated and untreated substrates of all concentrations and inoculums amount. On the 4th day of fermentation, the amount of ethanol was found to be smaller compared to other days of fermentation. This period of time could be adaptation period by yeasts where they can synthesize enzymes and essential metabolites that might be missing from their new environment so as to give more ethanol (Yates *et al.,* 2007).

The amount of ethanol, however, peaked on the 8th day of fermentation and found to decline thereafter (Table 3). At this period of time, yeast cells were dividing at maximum rate and able to convert fermentable compounds into ethanol. After 8th day of fermentation, ethanol production declined may be due to depletion of fermentable substrate or accumulation of toxic metabolites that may hinder the activity of yeast (Aikin *et* al., 2005).

Ethanol production measured at all time-point interval for all acid-treated substrates was significantly ($p<0.05$) higher than for acid untreated substrates (Table 3). This suggests that acid pre-treatment disintegrates the complex ligno-cellulosic structure and makes it accessible for fermentative enzymatic reaction (Sharma, 2007). For both acid pre-treated and untreated substrates, ethanol production increased with the amount of yeast inoculums. Likewise, substrate concentration affected ethanol production with the highest amount measured when 20g of the substrate

was fermented and declined thereafter suggesting that this amount of substrate is optimal. For every enzymatic reaction, there will be optimal substrate level to yield maximum product, and increasing the amount of substrate beyond optimal level may be limited by the amount of enzyme produced in the system (Grubb and Mawson, 1993); Reddy, 2006; Hoyer *et al*., 2009).

Table 3. Ethanol production from *L. camaras* leaves using S. cerevisiae

Substrate	Treatment	Ethanol produced at different fermentation period (%)			
		4th day	8th day	12th day	16th day
A	Treated	3.05 ± 0.08^{Od}	8.01 ± 0.08^{Ma}	4.85 ± 0.08^{Nb}	3.88 ± 0.1^{Nc}
	Untreated	2.93 ± 0.23^{Pd}	6.7 ± 0.25^{Oa}	4.03 ± 0.12^{Pb}	3.17 ± 0.25^{Pc}
B	Treated	$3.84\pm0.08^{M d}$	8.09 ± 0.09^{La}	5.71 ± 0.16^{Mb}	4.34 ± 0.14^{Mc}
	Untreated	3.37 ± 0.17^{Nd}	7.41 ± 0.02^{Na}	4.73 ± 0.17^{Ob}	3.62 ± 0.04^{Oc}
C	Treated	10.31 ± 0.10^{Bd}	18.04 ± 0.15^{Ca}	16.94 ± 0.17^{Cb}	15.57 ± 0.25^{Cc}
	Untreated	8.34 ± 0.27^{Dd}	16.28 ± 0.13^{Da}	15.38 ± 0.5^{Fb}	12.83 ± 0.3^{Gc}
D	Treated	12.43 ± 0.05^{Ad}	20.50 ± 0.47^{Aa}	18.68 ± 0.36^{Ab}	17.70 ± 0.06^{Ac}
	Untreated	9.53 ± 0.25^{Cd}	19.32 ± 0.31^{Ba}	16.14 ± 0.49^{Db}	15.45 ± 0.36^{Dc}
E	Treated	6.91 ± 0.11^{Fd}	15.93 ± 0.08^{Fa}	15.51 ± 0.13^{Eb}	13.65 ± 0.10^{Ec}
	Untreated	5.83 ± 0.11^{Hd}	15.77 ± 0.11^{Ga}	13.73 ± 0.16^{Hb}	11.32 ± 0.30^{Hc}
F	Treated	7.82 ± 0.15^{Ed}	18.30 ± 0.28^{Ca}	17.07 ± 0.15^{Bb}	15.63 ± 0.23^{Bc}
	Untreated	6.82 ± 0.13^{Gd}	16.58 ± 0.29^{Ea}	15.11 ± 0.36^{Gb}	13.24 ± 0.22^{Fc}
G	Treated	4.76 ± 0.1^{Jd}	11.99 ± 0.06^{Ja}	10.77 ± 0.15^{Jb}	7.25 ± 0.18^{Jc}
	Untreated	3.95 ± 0.07^{Ld}	11.08 ± 0.21^{Ka}	8.45 ± 0.36^{Lb}	5.66 ± 0.1^{Lc}
H	Treated	5.52 ± 0.27^{Id}	13.29 ± 0.28^{Ha}	11.44 ± 0.69^{Ib}	8.38 ± 0.36^{Ic}
	Untreated	4.5 ± 0.08^{Kd}	12.54 ± 0.21^{Ia}	8.91 ± 0.66^{Kb}	6.60 ± 0.21^{Kc}

means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different.

A= 10g + 0.5% yeast, B=10g + 1% yeast, C= 20g + 0.5% yeast, D= 20g + 1% yeast, E= 30g + 0.5% yeast, F= 30g + 1% yeast, G= 40g + 0.5% yeast and H= 40g + 1% yeast

3.2. Effect of Acid Pre-treatment, Substrate and Inoculum Concentrations and Fermentation Period on Reducing Sugar Yield

In both acid pre-treated and untreated substrates, the amount of reducing sugar decreased with increasing period of fermentation (Table 4). Reduction in the amount of reducing sugar with fermentation period may be ascribed to consumption of sugars by more number of yeast produced through time for grow and production of ethanol. This result agrees with increased ethanol production observed in this experiment, suggesting that the reduction in reducing sugar is due to its conversion to ethanol. Increasing inoculum concentration reduced the amount of reducing sugar. This is because the higher the amount of yeast in the fermenter means more consumption of reducing sugars. Substrate concentration affected ethanol production with the highest amount measured when 20g of the substrate was fermented and declined thereafter suggesting that this amount of substrate is optimal.

In agreement with the amount of ethanol produced, the amount of reducing sugar measured at all time-point interval for all acid-treated substrates was significantly ($p<0.05$) higher than for acid untreated substrates (Table 4). This suggests that acid pre-treatment disintegrates the complex ligno-cellulosic structure and makes it accessible for fermentative enzymatic reaction to be converted to ethanol (Aden *et al*., 2002; Sharma, 2007).

According to Saha *et* al. (2005), dilute acid pre-treatment has the advantage of not only solubilizing hemicelluloses, but also facilitates the hydrolysis of lignocelluloses material for conversion into sugar. Authors such as Demirbas (2005) and Hendricks and Zeeman (2009) ` previously reported that acid pre-treatment of lignocelluloses is important to break down lignin and increase the availability of sugar for microbes to grow on and convert it to ethanol. Rate of decrement of reducing sugar agrees with the amount of ethanol production, suggesting that the more is the reduction in reducing sugar means the more it is converted to ethanol by yeast This finding was in agreement with Alemayehu (2005). In addition to this Sirkar *et al.* (2008) reported that acid pre-treatment method was found to be optimal for better yield of fermentable sugars from lignocelluloses material.

Table 4. Reducing sugar concentration (mg/ml) measured at 540nm

Substrate yeast	Treatment	Reduced sugar observed from *Lantana camara* at 540nm (g/ml)			
		4th	8th	12th	16th
A	Treated	13.64±0.11^{Ha}	7.76±0.15^{Hb}	4.78±0.06^{Ec}	2.32±0.22^{Fd}
	Untreated	11.70±0.30^{Ma}	4.02±0.15^{Ob}	3.08±0.97^{Mc}	2.81±0.16^{Jd}
B	Treated	12.47±0.45^{La}	6.60±0.22^{Jb}	3.04±0.06^{Nc}	2.04±0.04^{Md}
	Untreated	10.61±0.31^{Pa}	3.77±0.21^{Pb}	2.18±0.25^{Pc}	1.94±0.08^{Pd}
C	Treated	20.79±0.38^{Aa}	12.41±0.16^{Ab}	6.59±0.18Ac	5.72±0.04^{Ad}
	Untreated	19.07±0.02^{Ca}	10.09±0.1^{Cb}	6.15±0.11^{Bc}	4.44±0.22^{Bd}
D	Treated	19.46±0.42^{Ba}	9.67±0.07^{Db}	4.56±0.2^{Fc}	2.66±0.11^{Id}
	Untreated	17.4±0.11^{Da}	7.8±0.1^{Gb}	3.97±0.21 ^{Hc}	2.27±0.05^{Kd}
E	Treated	16.13±0.11^{Ea}	10.14±0.04^{Bb}	5.26±0.1^{Cc}	4.41±0.21^{Cd}
	Untreated	12.83±0.35^{Ka}	4.83±0.09^{Mb}	3.6±0.29^{Jc}	3.1±0.19^{Gd}
F	Treated	14.23±0.27^{Fa}	8.48±0.1^{Fb}	3.83±0.24^{Ic}	2.18±0.06^{Jd}
	Untreated	11.59±0.29^{Na}	5.08±0.37^{Lb}	3.22±0.22^{Lc}	2.07±0.09^{Ld}
G	Treated	14.02±0.01^{Ga}	9.45±0.02^{Eb}	5±0.02^{Dc}	3.95±0.08^{Dd}
	Untreated	12.83±0.35^{Ka}	4.83±0.09^{Mb}	3.6±0.29^{Jc}	3.1±0.19^{Gd}
H	Treated	13.19±0.11^{Ia}	7.72±0.13^{Ib}	3.37±0.08^{Kc}	2.05±0.03Nd
	Untreated	11.36±0.14^{Oa}	4.15±0.08^{Nb}	2.79±0.11^{Oc}	2.01±0.01^{Od}

A= 10 g + 0.5% yeast, B=10g + 1% yeast, C= 20g + 0.5% yeast, D= 20g + 1% yeast, E= 30g + 0.5% yeast, F= 30g + 1% yeast, G= 40g + 0.5% yeast and H= 40g + 1% yeast

means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different.

3.3. Effect of Acid Pre-Treatment, Substrate and Inoculums Concentrations and Fermentation Period on Cell Density

Compared to the 8th and 12th days of fermentation, cell density was lower on the 4th and 16th days of fermentation in both acid pre-treated and untreated substrates (Table 5). Cell density was lower on the 4th day because yeast cells are on adaptation period and not yet dividing maximally. However, decrement on the 16th day of fermentation may be due to depletion of nutrients and/or accumulation of toxic metabolites that hinder growth of yeast cell. due to yeasts. Cell density peaked on the 8th day and this fact agrees with the highest amount of ethanol measured. The increasing ethanol production with increasing cell biomass indicated that the amount of yeast influenced ethanol production (Akin-Osanaiye *et al.,* 2005). Cell density appeared to increase with increasing substrate concentration, suggesting that the higher the substrate, the more is the required nutrients to sustain yeast population.

Table 5. Cell density (mg/ml) measured at 600nm in L. camara leaves

Substrate	Treatment	Cell density (mg/ml) at different fermentation period			
		4th day	8th day	12th day	16th day
A	Treated	0.76±0.01^{GHc}	2.69±0.25^{La}	2.17±0.17^{Jb}	0.52±0.03^{Id}
	Untreated	0.64±0.01^{Ic}	2.41±0.23^{Na}	2.09±0.15^{Kb}	0.42±0.03^{Jd}
B	Treated	1.46±0.1^{Cc}	3.28±0.13^{Da}	2.67±0.11^{Gb}	1.36±0.11^{Cd}
	Untreated	1.16±0.15^{Fc}	3.03±0.06^{Ga}	2.42±0.14^{Hb}	1.19±0.1^{Fd}
C	Treated	0.78±0.01^{Gd}	3.32±0.2^{Da}	2.85±0.09^{Eb}	1.39±0.16^{Cc}
	Untreated	0.75±0.01^{Gd}	2.95±0.07^{Ha}	2.64±0.11^{Fb}	1.17±0.17^{Fc}
D	Treated	1.78±0.01^{Ac}	3.65±0.08^{Aa}	3.33±0.17^{Ab}	1.60±0.04^{Ad}
	Untreated	1.56±0.02^{Bc}	3.37±0.09^{Ca}	3.13±0.11^{Bb}	1.4± 0.05^{Cd}
E	Treated	0.77±0.01^{Gd}	2.96±0.06^{Ia}	2.72±0.04^{Fb}	1.2± 0.16^{Ec}
	Untreated	0.73±0.02^{GHd}	2.79±0.15^{Ka}	2.39±0.11^{Hb}	1.03±0.08^{Lc}
F	Treated	1.6±0.06^{Bc}	3.49±0.04^{Ba}	3.15±0.15 ^{Bb}	1.46±0.07^{Bd}
	Untreated	1.39±0.04^{Dc}	3.15±0.1^{Ea}	2.97±0.01^{Db}	1.31±0.04^{Dd}
G	Treated	0.76±0.01^{Gd}	2.85±0.1^{Ja}	2.68±0.01^{Fb}	1.11±0.1^{Gc}
	Untreated	071±0.01^{Hd}	2.63±0.05^{Ma}	2.29±0.12^{Ib}	1±0.05^{Hc}
H	Treated	1.56±0.09^{Cc}	3.36±0.11^{Ca}	3.07±0.07^{Cb}	1.37±0.1^{Cd}
	Untreated	1.27±0.08^{Ec}	3.1±0.05^{Fa}	2.84±0.04^{Fb}	1.22±0.13^{Ed}

A=10g +0.5% yeast, B=10g +1% yeast, C=20g+0.5% yeast, D=20g+1% yeast, E=30g +0.5% yeast, F=30g +1% yeast, G=40g +0.5% yeast, H=40g +1% yeast. Means with the same letter (lower case) in the same row are not significantly different at $p<0.05$; means with the same letter (upper case) in the same column are not significantly different at $p<0.05$.

4. Summary, Conclusion and Recommendations

4.1. Summary

Ethanol is a clear liquid alcohol that is made by the fermentation of different biological materials. The main use of ethanol is as a motor fuel and fuel additive. With the aim of ethanol production from *Lantana camara*, a series of experiments were carried out with different substrate concentration (10gm, 20gm, 30g and 40gm) using batch digester up to 16th day. Though all substrate concentrations resulted in ethanol production starting from the fourth day of fermentation, 20g substrate was found to yield high amount of ethanol throughout the incubation period. Therefore, it can be considered as the optimum concentration. Moreover, it was observed that after 8th day of fermentation ethanol production decreased as time for fermentation increased. The reducing sugar concentrations were analyzed for acid pretreated and untreated substrate. The reducing sugars decreased gradually as the fermentation period increased. Moreover, on 4th day of fermentation period, the highest reducing sugar concentration (20.76mg/ml) was obtained from acid pretreated 20gram substrate mixed with 1% of yeast. The cell density was also observed and it was indicated that yeast biomass was higher at 8th day but go down there after.

5.2. Conclusion

The experiment, work shows that the bio-ethanol could be produced from *Lantana camara*. Bio-ethanol production from treated and untreated substrates was statistically significant at $p<0.05$. Among the different substrate concentration, fermentation days, treatment, inoculums concentration there is an effect on ethanol production. From different substrate concentrations and the experiment was carried out in thesis paper and then, the following conclusion are drown. The inoculums concentration also increases until it reached the optimum level for ethanol production. Bio-ethanol production increased slightly when initial substrate concentration increased. However, it was decreased, after 8th day of fermentation. This may be due to substrate limitation and decrease in cell biomass (yeast).

The study also treatment effect on ethanol production. Comparatively the reducing sugar utilization was more in pre-treated substrates than untreated ones. Reducing sugar and ethanol production was directly proportion. Cell density increasing at 8 days because of microbial growth. On the next 12th day and 16th day silently decreases. Due to cysto-toxicity of ethanol and limitation of available nutrient resource.

5.3. Recommendations

Based on the findings of the research, the following recommendations are expected from bio-ethanol production from *Lantana camara* leaves by batch culture using *S. cerevisiaeas:*

- It is also recommended to check the bio-ethanol quality of *Lantana camara* by Gas chromatography.
- Further study is very important to describe how absolute bio-ethanol can be produced from *Lantana camara* by using rotary evaporator, because it is difficult to make pure ethanol since there are other chemicals that can evaporate below the boiling point of ethanol (78^0c) and distillation by rotary evaporator produced only 95% pure alcohol.

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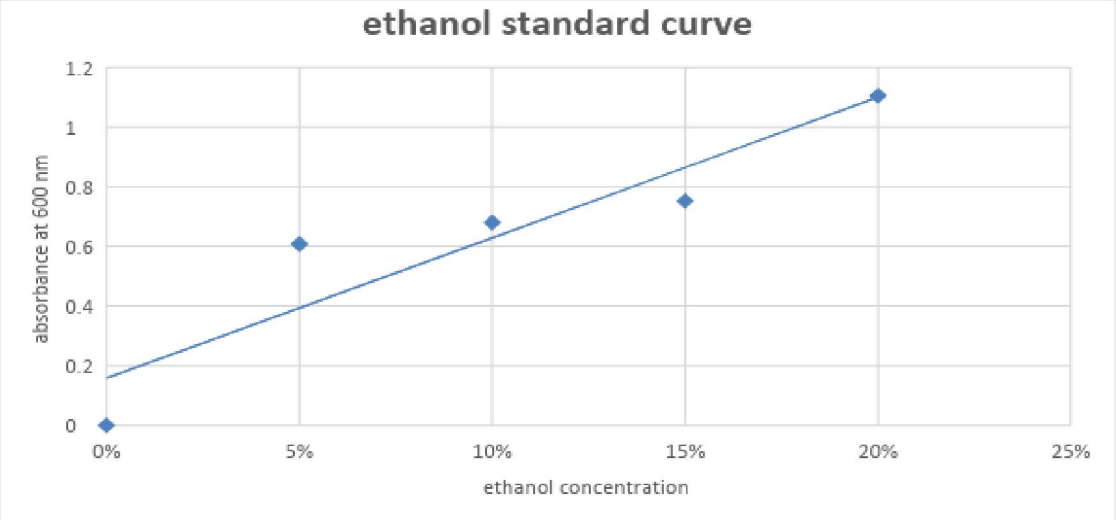
5. Appendix

List Of Figures In The Appendix

Appendix Table 1. Standard curve of ethanol

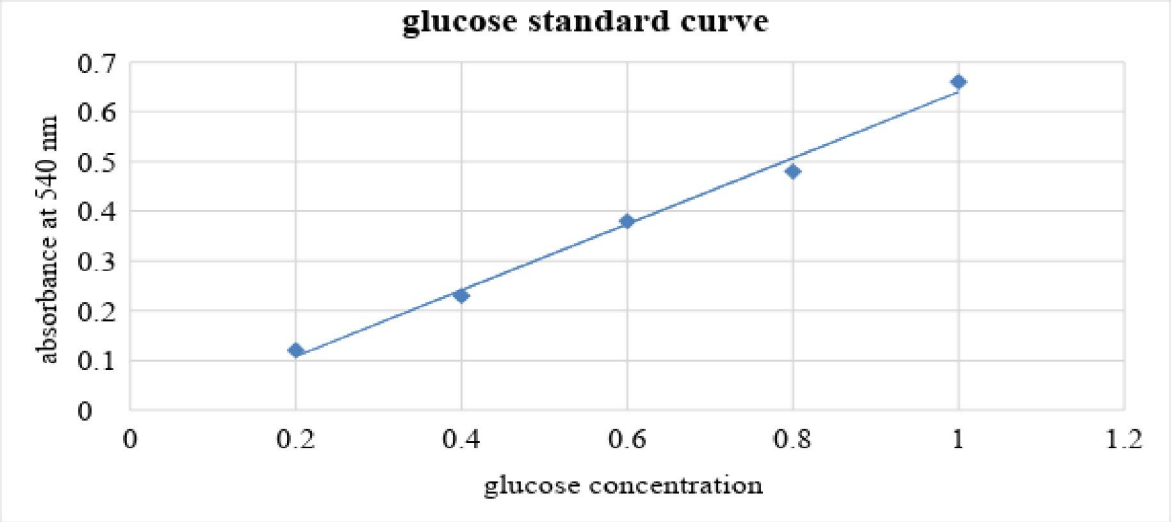
Test tube	Concentration	Absorbance
1	0%	0
2	5%	0.608
3	10%	0.68
4	15%	0.752
5	20%	1.105



Appendix Figure 1. Standard curve for determination of ethanol concentration

Appendix Table 2. Standard curve of glucose

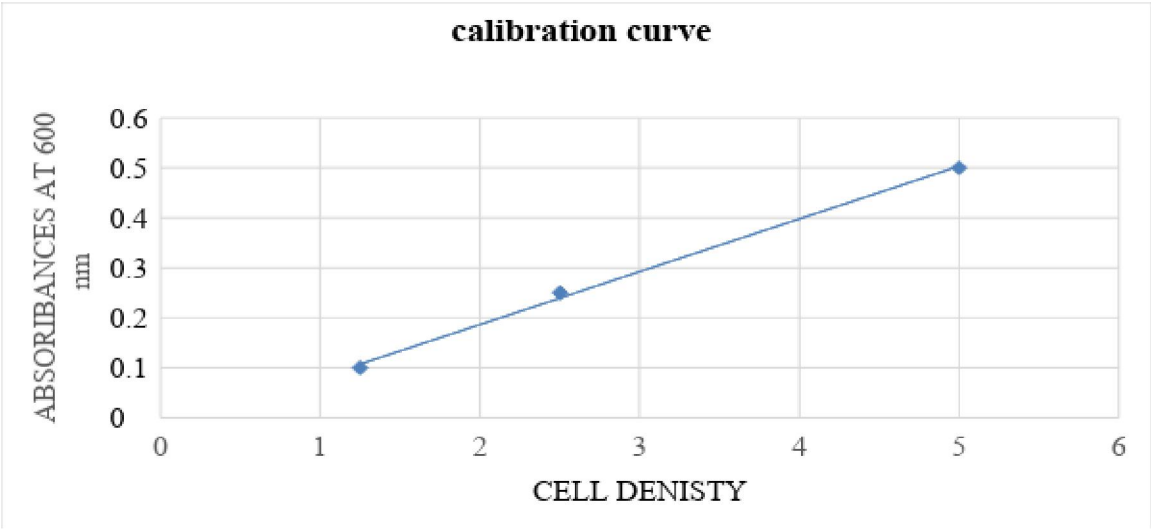
Test tube	Concentration	Absorbance
1	0.2	0.12
2	0.4	0.23
3	0.6	0.38
4	0.8	0.48
5	1	0.66



Appendix Figure 2. Standard curve for determination of glucose concentration

Appendix Table 3. Standard curve of cell density

Test tube	cell density	Absorbance
1	5.0	0.5
2	2.5	0.25
3	1.25	0.1



Appendix Figure 3. Calibration curve for determination of cell density

3/20/2019